http://www.expertreviews.org/

expert reviews
in molecular medicine

Ebola virus: new insights into disease aetiopathology and possible therapeutic interventions

Thomas W. Geisbert and Lisa E. Hensley

Ebola virus (EBOV) gained public notoriety in the last decade largely as a consequence of the highly publicised isolation of a new EBOV species in a suburb of Washington, DC, in 1989, together with the dramatic clinical presentation of EBOV infection and high case-fatality rate in Africa (near 90% in some outbreaks), and the unusual and striking morphology of the virus. Furthermore, there are no vaccines or effective therapies currently available. Progress in understanding the origins of the pathophysiological changes that make EBOV infections of humans so devastating has been slow, primarily because these viruses require special containment for safe research. However, an increasing understanding of the mechanisms of EBOV pathogenesis, facilitated by the development of new tools to elucidate critical regulatory elements in the viral life cycle, is providing new targets that can be exploited for therapeutic interventions. Notably, identifying factors triggering the haemorrhagic complications that characterise EBOV infections led to the development of a strategy to modulate coagulopathy; this therapeutic modality successfully mitigated the effects of EBOV haemorrhagic fever in nonhuman primates. This review summarises our current understanding of EBOV pathogenesis and discusses various approaches to therapeutic intervention based on our current understanding of how EBOV produces a lethal infection.

Thomas W. Geisbert (corresponding author)
Chief, Department of Viral Pathology and Ultrastructure, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011, USA. Tel: +1 301 619 4803; Fax: +1 301 619 2290; E-mail: tom.geisbert@amedd.army.mil

Lisa E. Hensley
Research Microbiologist, Virology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD 21702-5011, USA. Tel: +1 301 619 4808; Fax: +1 301 619 2290; E-mail: lisa.hensley@amedd.army.mil

Institute URL: http://www.usamriid.army.mil

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
21 SEP 2004	**N/A**	**-**

4. TITLE AND SUBTITLE
Ebola virus: new insights into disease aetiopathology and possible therapeutic interventions, Expert Reviews of Molecular Medicine 6:1 - 24

5a. CONTRACT NUMBER
5b. GRANT NUMBER
5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Geisbert, TW Hensley, LE

5d. PROJECT NUMBER
5e. TASK NUMBER
5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD

8. PERFORMING ORGANIZATION REPORT NUMBER
RPP-04-322

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)
11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES
The original document contains color images.

14. ABSTRACT
Ebola virus (EBOV) gained public notoriety in the last decade largely as a consequence of the highly publicised isolation of a new EBOV species in a suburb of Washington, DC, in 1989, together with the dramatic clinical presentation of EBOV infection and high case-fatality rate in Africa (near 90% in some outbreaks), and the unusual and striking morphology of the virus. Furthermore, there are no vaccines or effective therapies currently available. Progress in understanding the origins of the pathophysiological changes that make EBOV infections of humans so devastating has been slow, primarily because these viruses require special containment for safe research. However, an increasing understanding of the mechanisms of EBOV pathogenesis, facilitated by the development of new tools to elucidate critical regulatory elements in the viral life cycle, is providing new targets that can be exploited for therapeutic interventions. Notably, identifying factors triggering the haemorrhagic complications that characterise EBOV infections led to the development of a strategy to modulate coagulopathy; this therapeutic modality successfully mitigated the effects of EBOV haemorrhagic fever in nonhuman primates. This review summarises our current understanding of EBOV pathogenesis and discusses various approaches to therapeutic intervention based on our current understanding of how EBOV produces a lethal infection.

15. SUBJECT TERMS
Filovirus, Ebola Reston, Zaire, pathogenesis, review, therapy

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**24**	

Ebola virus (EBOV) infections are usually the most severe of those caused by the viruses of lethal haemorrhagic disease in humans. Clinical symptoms appear suddenly after an incubation period of 2 to 21 days (Ref. 1). Common presenting complaints include high fever, chills, malaise and myalgia (Refs 2, 3, 4, 5, 6, 7). As the disease progresses, there is evidence of multisystemic involvement, and manifestations include prostration, anorexia, vomiting, nausea, abdominal pain, diarrhoea, shortness of breath, sore throat, oedema, confusion and coma (Refs 2, 3, 4, 5, 6, 7). Abnormalities in blood coagulation and fibrinolysis are manifested as petechiae, ecchymoses, mucosal haemorrhages, and uncontrolled bleeding at venipuncture sites (Refs 2, 3, 4, 5, 6, 7); however, massive loss of blood is atypical and, when present, is largely restricted to the gastrointestinal tract. In fact, even in these cases, blood volume loss is insufficient to account for death. The presence of a maculopapular rash is a prominent feature (Refs 2, 3, 4, 5, 6, 7), but is not pathognomonic for EBOV haemorrhagic fever (HF). Fulminant EBOV infection typically evolves to shock, convulsions, and, in most cases, diffuse coagulopathy (Refs 2, 3, 4, 5, 6, 7). Death usually occurs 6–9 days after the onset of clinical symptoms. It should be noted that evidence of asymptomatic EBOV infection was documented in a small group of individuals during a recent outbreak (Ref. 8), but the clinical and epidemiological relevance of this observation is uncertain.

Epidemiology

EBOV was first recognised during near-simultaneous explosive outbreaks in 1976 in small communities in the former Zaire [now the Democratic Republic of the Congo (DRC)] (Ref. 6) and Sudan (Ref. 5). There was significant secondary transmission through reuse of unsterilised needles and syringes, and nosocomial contacts. These independent outbreaks involved serologically distinct viral species: Zaire EBOV (ZEBOV) and Sudan EBOV (SEBOV). The ZEBOV outbreak involved 318 cases and 280 deaths (88% mortality), while the SEBOV outbreak involved 284 cases and 151 deaths (53% mortality). Since 1976, EBOV has appeared sporadically in Africa, causing several small to mid-size outbreaks between 1976 and 1979. In 1995, there was a large epidemic of ZEBOV HF involving 315 cases, with an 81% case fatality rate, in Kikwit, a community in the former Zaire (Ref. 1). Meanwhile, between 1994 and 1996, there were smaller outbreaks caused by ZEBOV in Gabon (Ref. 9). More recently, Uganda, Gabon and the DRC suffered large epidemics of viral HF attributed to EBOV. The most recent outbreak in the DRC also involved a catastrophic decline in populations of great apes, which are thought to have a role in transmission to humans (Ref. 10).

In 1989, a third species of EBOV, Reston EBOV (REBOV), appeared in Reston, VA, USA, in association with an outbreak of viral HF among cynomolgus monkeys (*Macaca fascicularis*) imported to the USA from the Philippine Islands (Ref. 11). Hundreds of monkeys were infected (with high mortality) in this episode, but no human cases occurred (although four animal caretakers seroconverted without overt disease). Epizootics in cynomolgus monkeys recurred at other facilities in the USA and Europe through 1992, and again in 1996. A fourth species of EBOV, Ivory Coast EBOV (ICEBOV), was identified in Côte d'Ivoire in 1994; this species was associated with chimpanzees and only one (nonfatal) human infection was identified (Ref. 12).

Very little is known about the natural history of EBOV. Implication of animal reservoirs and arthropod vectors has been aggressively sought without success. (See Ref. 13 for a more detailed discussion of the epidemiology of EBOV.)

Taxonomy

EBOV belongs to the family Filoviridae, which comprises filamentous, enveloped, nonsegmented, negative-sense RNA viruses (reviewed in Ref. 13; see also http://www.ncbi.nlm.nih.gov/ICTV). The family Filoviridae is divided into two genera: *Marburgvirus* and *Ebolavirus*. The *Marburgvirus* genus contains a single species: Lake Victoria marburg virus (LVMARV). The *Ebolavirus* genus consists of the four species of EBOV discussed above: ZEBOV, SEBOV, REBOV and ICEBOV.

EBOV: structure and protein functions

EBOV particles contain an ~19 kb, single negative-stranded, linear RNA genome that is noninfectious. The genome encodes seven structural proteins, with a gene order of: 3' leader, nucleoprotein (NP), virion protein (VP) 35 (VP35), VP40, glycoprotein (GP), VP30, VP24, polymerase L protein, and 5' trailer (Ref. 14). Four of these proteins – NP, VP30, VP35 and L – associate with the genomic RNA in a ribonucleoprotein complex,

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

while the three remaining proteins (GP, VP24 and VP40) are associated with the membrane. GP is the surface glycoprotein that forms the spikes on the virion and is the effector for receptor binding and membrane fusion (Refs 15, 16). GP is synthesised as a precursor molecule, GP_0; this is postranslationally cleaved by furin or a furin-like endoprotease into two subunits – GP_1 and GP_2 – which are linked by disulphide bonds to form a heterodimer (Refs 17, 18). Homotrimers of GP_1–GP_2 form the virion spikes. The primary gene product of the EBOV GP gene is not the GP, but rather a smaller nonstructural secreted GP (sGP), which is efficiently released from infected cells (Refs 19, 20). The function of sGP has not been fully delineated, but it appears that expression of sGP might protect against cytotoxicity (Ref. 21). VP40 functions as a matrix protein and is responsible for the formation of the filamentous particles (Ref. 22). VP24 is a minor viral protein whose functions remain unknown, but recent data indicate that VP24 possesses structural features consistent with a function as a viral matrix protein and suggest that VP24 might have a role in viral assembly and budding (Refs 23, 24, 25).

EBOV receptors and entry mechanism

The viral entry process consists of three sequential stages: attachment, co-receptor binding and fusion. Viral fusion can occur at the host cell plasma membrane or viruses can exploit the host cell's endocytic machinery to access the cytoplasm, depending on the characteristics of the viral fusion protein. The entry of filoviruses into host cells has not been fully described, but is not thought to occur by direct fusion with the plasma membrane (Ref. 26). The endocytic routes by which extracellular ligands, including viruses, are internalised into the cell include clathrin-coated vesicles, caveolae, macropinosomes, and other pathways that presently are poorly characterised. One study suggested that filoviruses use caveolae to gain entry into host cells (Ref. 27); however, subsequent work by others showed that caveolae are not required for EBOV entry (Ref. 28). It is known that EBOV entry is mediated by the binding of transmembrane viral GP to cell-surface receptors (Ref. 15). Currently, four different types of cell-surface receptors have been proposed to play a role in EBOV entry: the β1 integrin receptors (Ref. 29); the folate receptor α (Ref. 30); the dendritic-cell-specific intercellular adhesion molecule (ICAM)-3-grabbing nonintegrin (DC-SIGN) and DC-SIGN-related (DC-SIGNR) factors (Refs 31, 32, 33); and a human macrophage galactose- and *N*-acetylgalactosamine-specific C-type lectin (hMGL) (Ref. 34). Although folate receptor α was originally thought to play an important role in EBOV entry (Ref. 30), recent studies show that this receptor is not required for EBOV infection (Refs 28, 35), questioning its role as a key factor in EBOV entry. As EBOV has a broad cell tropism, infecting a wide range of cell types (Refs 36, 37, 38, 39, 40), it is highly likely that EBOV exploits several molecules for entry into host cells. In support of this view, Takada and colleagues recently proposed that EBOV uses a variety of different C-type lectins for efficient entry into host cells (Ref. 34).

EBOV replication and transcription

EBOV replication and transcription is described only briefly here; for a more detailed account, see relevant book chapters on this subject (Refs 13, 41). Replication and transcription of EBOV occurs exclusively in the host cell cytoplasm. Because the negative-strand EBOV RNA genomes cannot be employed as templates by the DNA-dependent RNA polymerases of the host cell, EBOV encodes its own RNA-dependent RNA polymerase, the L protein. The L protein is thought to control all catalytic functions that are required for replication and transcription. Fusion of the viral membrane with the host cell membrane releases the EBOV ribonucleoprotein complexes (the viral RNA and the proteins NP, VP35, VP30 and L) into the cytoplasm. The RNA genome is then transcribed into mRNAs to generate the EBOV proteins, while replication leads to synthesis of a replicative intermediate. This full-length antigenomic RNA, which is a copy of the negative-strand RNA genome, then functions as a template for EBOV RNA genome synthesis. Lastly, the nascent ribonucleoprotein complexes are assembled with EBOV structural proteins at the plasma membrane or intracellular membranes.

Animal models of EBOV HF

Animal models have been invaluable for studying the pathogenesis of numerous infectious diseases as well as for testing the efficacy of experimental prophylactic and therapeutic vaccine and/or drug regimens. The development of animal models for EBOV HF has been particularly challenging because the pathophysiology of human EBOV HF

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

has not been clearly defined. This shortcoming is primarily due to the limited number of cases being managed in a medical setting equipped for both safe and exhaustive clinical laboratory evaluations.

Mice, guinea pigs, and several nonhuman primate species have been employed to model EBOV HF (reviewed in Refs 42, 43). In addition to the encumbrances caused by a paucity of human data, comparison of the disease pathogenesis among these groups is difficult because of differences in age, route of infection, dose administered, and the nature of the challenge virus itself. As an example, the challenge dose appears to have a profound effect on the course of disease in nonhuman primates. Cynomolgus macaques exposed by intramuscular injection with a low challenge dose of ZEBOV [10 plaque-forming units (pfu)] succumbed to infection 8–12 days after challenge (mean = 9.8 days), whereas cynomolgus monkeys exposed by intramuscular injection to a high dose (1000 pfu) of the exact same ZEBOV isolate died 5–8 days after challenge (mean = 6.3 days) (Refs 44, 45). In human cases, route of infection ostensibly affects the disease course and the outcome. The mean incubation period for cases of ZEBOV known to be due to injection was 6.3 days, versus 9.5 days for contact exposures (Ref. 46). Moreover, the case-fatality rate in this 1976 ZEBOV outbreak was 100% (85 of 85) in cases associated with injection compared with ~80% (119 of 149) in cases of known contact exposure (Ref. 46). General features of disease pathogenesis associated with ZEBOV infection in mice, guinea pigs, and nonhuman primates are compared with human ZEBOV infection in Table 1.

Rodents

Guinea pigs and mice have been the primary rodent models employed to study EBOV HF (Refs 47, 48, 49, 50). Serial adaptation is required to produce lethal disease in rodents because isolates obtained from fatal primate infections rarely produce severe illness in rodents on initial exposure. Although rodents have unquestionably served as important models of EBOV infection, we recently showed that rodent models of EBOV HF are not ideal for studying human EBOV HF (Ref. 44); also, others have suggested that guinea pigs are inadequate for evaluating the pathogenesis of human EBOV HF (Ref. 47). More specifically, mice do not exhibit the coagulation abnormalities that characterise primate EBOV infections (Refs 44, 51). The development of coagulopathy in EBOV-infected guinea pigs is uncertain, with findings varying among studies (Refs 44, 48, 50, 51). Also, bystander lymphocyte apoptosis, which is associated with human and nonhuman primate EBOV infections (Ref. 52), has yet to be reported in EBOV-infected mice or guinea pigs (Refs 49, 50, 53), although a systematic investigation is needed to determine the incidence of lymphocyte apoptosis in the rodent models. Although the value of rodents for evaluating the efficacy of immune- and/or coagulation-modulating therapies is of course in doubt, these observations do not imply that rodent models of EBOV infection have no utility whatsoever. Rodents can serve an important role for evaluating the antiviral efficacy of candidate therapies against EBOV infection, and genetically engineered mice clearly have utility for evaluating specific host–pathogen interactions.

Nonhuman primates

Not unexpectedly, clinical disease and related pathology in nonhuman primates infected with EBOV appear to closely resemble features described for human EBOV HF. Several primate species have been employed to model ZEBOV HF, including African green monkeys (*Chlorocebus aethiops*) (Refs 39, 54, 55, 56, 57), cynomolgus macaques (Refs 44, 45, 55, 58, 59, 60, 61, 62, 63), rhesus macaques (*Macaca mulatta*) (Refs 38, 44, 54, 64, 65), and hamadryad baboons (*Papio hamadryas*) (Refs 56, 57, 66, 67, 68). Similar pathological features of ZEBOV infection have been documented among these species; however, a few pathological features differ. Most notably, African green monkeys do not present with the macular rash that is characteristic of disease in macaques and baboons (Refs 38, 54, 55, 61, 65, 66); importantly, this rash also appears to be a prominent feature of human disease (Refs 2, 5, 6). Lymphoid depletion and impairment of the microcirculation as evidenced by formation of fibrin thrombi in visceral organs is seen to various degrees. Species-specific differences in the appearance of coagulopathies have been reported: fibrin deposition is seen in vessels of African green monkeys whereas haemorrhages are seen in baboons (Ref. 56). Species-specific differences in coagulopathy among cynomolgus and rhesus macaques experimentally infected with ZEBOV have not been observed (Ref. 63).

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

Table 1. Comparison of disease pathogenesis of Ebola virus infection in animal models and humans[a]

Element / Feature	Mouse	Guinea pig	Macaque	Human
Time to death[b]	5–7 days	8–12 days	5–10 days	Up to 30 days
Fever	No	Yes	Yes	Yes
Anorexia	Yes	Yes	Yes	Yes
Peak viraemia	7.9 log10 pfu/ml	5.2 log10 pfu/ml	6.9 log10 pfu/ml	6.5 log10 pfu/ml
Thrombocytopaenia	Yes	Yes	Yes	Yes
Bleeding[c]	None	Rare	Occasional	Occasional
Macular rash	No	No	Yes	Yes
DIC (biochemical evidence)	None	Equivocal	Yes	Yes
DIC (fibrin deposits)	None	Few	Abundant	NE
Lymphopaenia	Equivocal	Yes	Yes	Yes
Lymphocyte apoptosis	NE	NE	Yes	Yes
Permissive host cells	Monocytes, macrophages, dendritic cells, fibroblasts, hepatocytes, adrenal cortical cells, endothelial cells, epithelial cells	Monocytes, macrophages, dendritic cells, fibroblasts, hepatocytes, adrenal cortical cells, endothelial cells, epithelial cells	Monocytes, macrophages, dendritic cells, fibroblasts, hepatocytes, adrenal cortical cells, endothelial cells, epithelial cells	Monocytes, macrophages, dendritic cells, fibroblasts, hepatocytes, endothelial cells, epithelial cells
Cytokines/chemokines (increased circulating levels)	MCP-1, TNF-α	NE	IFN-α, IL-6, IL-18, MIP-1α, MIP-1β, MCP-1, TNF-α	IFN-α, IL-2, IL-6, IL-10, TNF-α

[a] The table lists symptoms and characteristics of disease pathogenesis observed during ZEBOV infection of mice, strain 13 guinea pigs, nonhuman primates of the genus *Macaca*, and humans (summary of data from Refs 1, 2, 3, 6, 7, 8, 13, 38, 40, 42, 43, 44, 45, 46, 49, 50, 51, 52, 53, 55, 58, 59, 60, 61, 62, 63, 64, 65, 76, 77, 87, 96, 159).
[b] For animal models of ZEBOV HF, the time to death after experimental infection varies among studies and largely depends on the dose of ZEBOV employed. For human cases, information regarding time to death is difficult to assess because dose, route of exposure, and time of exposure are often unknown. In cases where route and time of exposure were documented, disease course appears to be more rapid in cases associated with injection than in cases of known contact exposure.
[c] Bleeding manifestations in nonhuman primates and humans are infrequent and include bleeding of the gums, haematemesis, bleeding from the rectum and/or bloody stools, haematuria, epistaxis, and bleeding at puncture sites.
Abbreviations: DIC, disseminated intravascular coagulation; IFN, interferon; IL, interleukin; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein; NE, not systematically evaluated; TNF, tumour necrosis factor.

Few studies have evaluated the pathogenesis of SEBOV in nonhuman primates (Refs 55, 69, 70). The disease course in rhesus and cynomolgus macaques appears slower (by several days) than

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

that seen in ZEBOV infections (Refs 55, 69), and rates of survival appear consistent with human disease. SEBOV infection was not lethal in a small cohort of African green monkeys, nor was REBOV (Ref. 55). Similar to SEBOV, and unlike ZEBOV, the disease course in REBOV-infected cynomolgus monkeys is protracted (Ref. 71). Bystander lymphocyte apoptosis is proposed to be the mechanism of lymphoid depletion in nonhuman primates experimentally infected with REBOV as well as ZEBOV (Ref. 52). Little is known about the pathogenesis of ICEBOV in nonhuman primates, apart from its high lethality in chimpanzees (*Pan troglodytes verus*) (Ref. 72).

Coagulopathy is a prominent feature of EBOV infection of nonhuman primates. Some reviewers have argued that fibrin deposition is not ubiquitous in EBOV-infected primates, citing original studies (e.g. Refs 56, 57) reporting that both viral strain and nonhuman species can affect the prominence of fibrin deposits. However, the appearance of fibrin deposits is only one of several indicators of a dysregulated coagulation response. Other indicators of coagulopathy include consumption of clotting factors, increases in clotting times, increases in levels of fibrin degradation products, and thrombocytopaenia. A more extensive review of previous ZEBOV studies in nonhuman primates reveals evidence of coagulopathy in nearly every case, although those correlates can vary with species. For example, for ZEBOV-infected baboons, dramatic changes were noted in blood-clotting parameters, including marked increases in fibrin degradation products, but fibrin deposits were not a prominent feature (Ref. 57). This finding conclusively shows that elevated levels of fibrin were being formed at some point during the course of infection.

Pathogenesis of EBOV HF: the host response to infection

Understanding the kinetics of host–pathogen relationships, and identifying critical pathogenetic processes, are important for the rational development of therapeutic interventions. A model representing our current understanding of EBOV pathogenesis in primates is shown in Figure 1. EBOV infection of humans and nonhuman primates is characterised by marked lymphopaenia and severe degeneration of lymphoid tissues (Refs 13, 61), and defects in the coagulation system. With a view to understanding these events, this section reviews the interactions between EBOV and the cells of lymphoid tissues, such as monocytes/macrophages, lymphocytes and dendritic cells, as well as cells that are important in maintaining the coagulation system, such as endothelial cells.

EBOV and monocytes/macrophages

The predilection of EBOV for monocytes/macrophages and their importance in EBOV pathogenesis are well documented (Refs 37, 40, 61, 73, 74). Recently, during a temporal study in nonhuman primates, monocytes/macrophages were shown to be among the cells initially infected (Ref. 61). As monocytes/macrophages are usually the cells that elicit the response cascade in the acute phase of inflammation (Ref. 75), their early infection represents an effective strategy for evading the host defence system as well as facilitating dissemination of the virus. EBOV infection of mononuclear phagocytes triggers a cascade of events involving cytokines/chemokines and oxygen free radicals (Refs 74, 76); it is thought that the consequence of these events, rather than direct viral infection, causes much of the observed pathology (Refs 73, 74, 76). Recent studies showed that EBOV infections might be further exacerbated by upregulation of anti-apoptotic genes – neuronal apoptosis inhibitory protein (NAIP) and cellular inhibitor of apoptosis protein 2 (cIAP2) – in ZEBOV-infected monocytes/macrophages (Ref. 61). This suggests that EBOV has evolved an additional mechanism to resist host defenses by inducing these protective transcripts in cells that it infects.

EBOV and lymphocytes

Despite the significant lymphocyte destruction associated with EBOV infections, lymphocytes do not support production of progeny virus (Refs 37, 52). Extensive lymphocyte apoptosis appears to be critical to the pathogenesis of EBOV in humans and nonhuman primates (Refs 52, 77). Recently, we showed that apoptosis of bystander lymphocytes, previously described in end-stage tissues, occurred early in the disease course in intravascular and extravascular locations (Ref. 61). Use of fluorescent caspase inhibitors to detect apoptosis indicated induction of apoptosis primarily among the $CD8^+$ and $CD16^+$ subsets of circulating lymphocytes (Refs 61, 78). The mechanism(s) underlying lymphocyte apoptosis has been unclear but is likely to be induced through multiple pathways. Analysis of

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

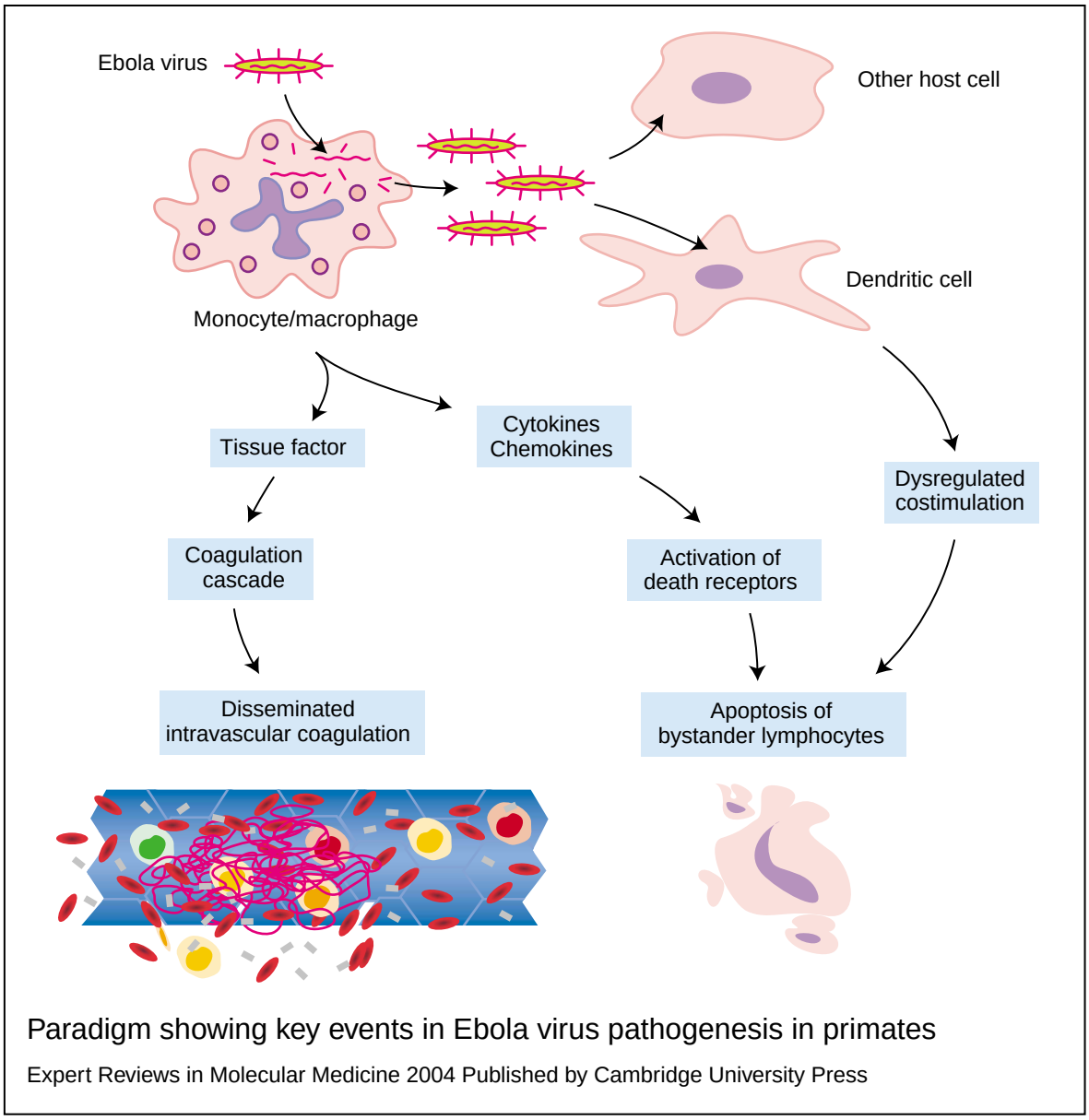

Figure 1. Paradigm showing key events in Ebola virus pathogenesis in primates. Ebola virus (EBOV) infection of monocytes/macrophages and dendritic cells appears central to much of the observed pathology. EBOV infection induces monocytes/macrophages to release a multitude of soluble factors including tissue factor and cytokines/chemokines, triggering a host of downsteam effects. Tissue factor leads to activation of the coagulation cascade, fibrin formation and development of disseminated intravascular coagulation. The release of cytokines/chemokines indirectly contributes to apoptosis of bystander lymphocytes by upregulating a variety of genes with pro-apoptotic functions. In addition, EBOV-induced dysfunction of dendritic cells likely impairs costimulatory signals important for rescuing activated T cells. The end result of these events is loss of homeostasis and dysregulation of the host immune response.

peripheral blood mononuclear cell (PBMC) gene expression in EBOV-infected cynomolgus monkeys showed temporal increases in tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and Fas transcripts, revealing possible pro-apoptotic mechanisms (Refs 61, 76). In addition, bystander lymphocyte apoptosis might be associated with dysfunction of dendritic cells induced by EBOV infection (discussed below).

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

EBOV and dendritic cells

In a recent temporal study in nonhuman primates, dendritic cells in lymphoid tissues were identified as early and sustained targets of ZEBOV, indicating their important role in the immunosuppression characteristic of EBOV infections (Ref. 61). This finding is of particular importance as others have shown that ZEBOV infects human monocyte-derived dendritic cells and impairs their function (Ref. 79). Specifically, monocyte-derived dendritic cells exposed to ZEBOV failed to secrete pro-inflammatory cytokines, did not upregulate costimulatory molecules including B7-1 and B7-2, and stimulated T cells poorly. Apoptosis of bystander lymphocytes during EBOV infections might result from the lack of costimulatory signals or from the engagement of death receptors or ligands such as Fas or TRAIL. As an example, dendritic cells prevent Fas-mediated T-cell apoptosis through costimulatory rescue signals (Ref. 80). Therefore, it is possible that EBOV-induced dysfunction of dendritic cells impairs costimulatory signals important for both rescue of activated T cells and/or for the proper development of T-cell responses. In addition, the rapid induction of TRAIL, and possibly Fas, in ZEBOV-infected macrophages and dendritic cells (Ref. 76) suggests that these might be key factors in the observed bystander apoptosis of lymphocytes in EBOV-infected nonhuman primates.

EBOV and endothelial cells

Recent studies suggested that the EBOV GP is the main determinant of vascular cell injury and consequently that direct EBOV-replication-induced structural damage of endothelial cells triggers the haemorrhagic diathesis (Refs 81, 82). Dysregulation of endothelial cell functions can cause a wide range of vascular effects that lead to changes in vascular permeability or haemorrhage (Refs 83, 84). Vascular damage can be induced by immunological mechanisms and/or by direct infection of the vascular tissue. Several microbial diseases are characterised by severe vascular lesions attributed to direct microbial-replication-induced damage to endothelial cells. For example, intracellular replication of *Rickettsia rickettsii*, the aetiological agent of Rocky Mountain spotted fever, directly induces lethal injury to host endothelial cells, causing pathophysiological changes including thrombosis, haemorrhage and vasculitis (Ref. 85). Another example is Nipah virus infection, where a systemic vasculitis with extensive thrombosis was attributed to infection, damage and necrosis of endothelial cells (Ref. 86). The aetiology of the haemorrhagic diatheses in fatal cases caused by the filoviruses MARV and EBOV was searched for in tissues from initial outbreaks in 1967 and 1976, respectively, but no vascular lesions were identified (Ref. 87). Nonetheless, there has been much speculation that EBOV-replication-induced structural damage of endothelial cells triggers the haemorrhagic diathesis.

In a recent study, and consistent with the original histology observations of Murphy in fatal human cases (Ref. 87), it was demonstrated that ZEBOV infection of endothelial cells does not extensively disrupt the architecture of the vascular endothelium in ZEBOV-infected cynomolgus monkeys (Ref. 62). Although ZEBOV replicated in endothelial cells of these animals, endothelial cell infection was only seen focally at late stages of disease, after the onset of the haemorrhagic abnormalities that characterise EBOV HF (Refs 61, 63). Although ultrastructural evidence of endothelial cell activation and disruption was observed, it is likely that the vasoactive effects on endothelial cells are mediated indirectly because these changes were not associated with the presence of intracytoplasmic EBOV antigens. Additionally, these data showed that while ZEBOV is capable of replicating in microvascular and macrovascular human endothelial cells in vitro, replication does not directly induce cytopathology (Ref. 62).

The subject of GP-mediated cytotoxicity and its relevance in pathogenesis is a highly controversial topic. Yang et al. reported that GP expression caused cell death (Refs 81, 82), whereas subsequent work by other groups showed that most of the detached cells (>90%) were viable, suggesting that expression of GP interferes with cell attachment but does not trigger cell death (Refs 88, 89). Another group reported that the cytotoxic effect of GP is associated with its level of expression (Ref. 21). In this study, using a novel reverse genetics system, Volchkov et al. generated a mutant virus in which the editing site of the GP gene was removed (Ref. 21). The mutant virus no longer expressed sGP. Notably, the mutant was significantly more cytotoxic than wild-type virus, showing that cytotoxicity caused by GP is downregulated by EBOV through transcriptional RNA editing and expression of sGP. Thus, the

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

authors postulated that transcriptional editing of the GP gene might play an important role in EBOV pathogenesis by restricting cytotoxicity and thereby increasing viral loads and promoting spread. The apparent protective effect of transcriptional editing and sGP is consistent with results detailed above showing that EBOV replication does not directly induce cytolysis in endothelial cells in vivo (Ref. 62).

Effect of the pro-inflammatory response

Cytokines and chemokines are soluble proteins that are generated and secreted in response to an assortment of attacks on a host organism, including microbial infection. Cytokines and chemokines function in a pleiotropic manner, acting on many different types of cells to modulate the host's immune response. While cytokines and chemokines typically apply their antimicrobial actions locally, for example in areas of infection, cytokines and chemokines might also act systemically, and they commonly induce many of the symptoms of infection (e.g. fever, myalgia). When present in high concentrations, cytokines and chemokines can have toxic or even lethal effects. Indeed, studies of septic shock have associated abnormal production of pro-inflammatory cytokines with disease severity and fatal outcome (Refs 90, 91, 92). For a detailed discussion of the clinical implications of dysegulated immune responses, see review articles on this subject (Refs 93, 94, 95).

Primate EBOV infections are characterised by a dysregulation of normal host immune responses. Increased levels of interleukin (IL)-10 have been associated with fatality in previous ZEBOV outbreaks (Refs 77, 96). One of these studies associated increased levels of interferon (IFN)-α with fatalities (Ref. 96), whereas the other study did not detect IFN-α, IL-1β, IL-8 or IL-12 (Ref. 77). Moderate levels of IL-6 and TNF-α were associated with fatal infection in the days before death in the Gabon study (Ref. 77), and high levels of TNF-α, but not IL-6, were associated with fatality in the Kikwit investigation (Ref. 96). In ZEBOV-infected nonhuman primates, increased circulating levels of IFN-α, IL-6, monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α and MIP-1β at the early to mid-stages of disease, and IFN-β, IL-18 and TNF-α at later stages of disease, were observed, whereas increased levels of IFN-γ, IL-4, IL-8, IL-10 or IL-12 were not detected (Ref. 61). In other studies, increased levels of IL-6, TNF-α, IFN-γ, IL-2, IL-4, IL-8, IL-10, granulocyte–monocyte colony-stimulating factor (GM-CSF), MCP-1, MIP-1β and RANTES (for 'regulated on activation, normal T cells expressed and secreted') were detected in nonhuman primates naturally infected with REBOV, and the increase in circulating cytokines was associated with an increase in circulating viral antigen (Ref. 97). However, the contribution of a second virus, simian HF virus, demonstrated in nearly all REBOV outbreaks to date, remains unknown and potentially confounds these results (Refs 11, 98).

The ability of EBOV to modulate directly the host pro-inflammatory response has yet to be fully delineated. The EBOV protein VP35 reportedly functions as a type I IFN (IFN-α/β) antagonist (Refs 99, 100, 101). Recent studies show that VP35 prevents IFN regulatory factor (IRF-3) activation by inhibiting phosphorylation, and it is likely that VP35 prevents EBOV-induced transcription of IFN-β (Ref. 100). Other studies suggest that VP24 expression might also interfere with type I IFN signalling (Ref. 101). Although not fully understood, the type I IFN response appears to be a critical determinant of EBOV pathogenicity. Studies performed in mice show that host resistance to ZEBOV infection is mediated by the type I IFN response (Ref. 102). Notably, mice lacking the IFN-α/β receptor died when challenged with a wild-type strain of ZEBOV that is not lethal in immunocompetent mice. Furthermore, treatment of immunocompetent mice with anti-IFN-α/β antibodies markedly accelerated the course of ZEBOV infection when animals were challenged with a lethal, adapted strain of ZEBOV.

Dysregulation of the coagulation system

Disseminated intravascular coagulation (DIC) is a syndrome characterised by systemic intravascular activation of coagulation leading to widespread deposition of fibrin in the vasculature, which contributes to the development of multiple organ failure (reviewed in Refs 103, 104). DIC is associated with both bleeding and thrombotic abnormalities, and, in fact, widespread thrombosis and bleeding commonly occur simultaneously. Although DIC is often viewed as a prominent manifestation of EBOV infection in primates, the presence of DIC in human filoviral infections has been a controversial topic; cultural mores and logistical problems have hampered

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

systematic studies. Fibrin deposition has been documented at autopsy for MARV (Ref. 87); furthermore, clinical laboratory data suggest that DIC is an important feature of human EBOV HF (Refs 2, 6). The coagulation picture is much clearer for nonhuman primates. Numerous studies have shown histological and biochemical evidence of DIC syndrome during EBOV infection in a variety of nonhuman primate species (Refs 37, 38, 39, 44, 51, 55, 61, 63, 64, 65). The mechanism(s) for triggering the coagulation abnormalities has not been fully delineated. New studies have begun to shed some light on the pathogenesis of coagulation system dysregulation and suggest that development of coagulation abnormalities might occur much earlier than previously thought. Although it is likely that the coagulopathy seen in EBOV HF is caused by multiple factors, particularly during the later stages of disease, recent data strongly implicate tissue factor (TF) expression/release from EBOV-infected monocytes/macrophages as key a factor that induces the development of coagulation irregularities seen in EBOV infections (Ref. 63).

Clinical implications/applications

There are no known effective treatments for human filoviral infections. At this time, treating patients infected with EBOV essentially consists of intensive supportive care. Progress to develop therapies/treatments has been encumbered primarily because these viruses require special containment (Biosafety Level 4) for safe research, but also as a result of the sporadic and transitory nature of filoviral outbreaks and confinement to remote geographic locales. Nonetheless, there have been a number of attempts in clinical settings to improve the status of infected patients, and several strategies have been developed and tested in animal models (Table 2). The treatment strategies are best analysed when broken down into the themes of direct antiviral approaches versus strategies to modulate the host immune response.

Antiviral approaches

Neutralisation of virus

For EBOV, passive therapy was first used to treat a laboratory worker who also received IFN (Ref. 105); he survived, but the role of immune plasma in his recovery was not clear. Several Russian laboratory workers potentially exposed to EBOV were treated with anti-EBOV goat IgG and IFN (Ref. 68); the workers survived, but again the role of the IgG in their recovery was uncertain. More recently, transfusion of convalescent-phase whole blood to infected patients in the 1995 Kikwit outbreak was anecdotally described as conferring increased survival on treated patients (Ref. 106), but other explanations for survival of seven of the eight patients have been proposed (Refs 106, 107). Of particular concern is the observation that the patient who died was treated 4 days after the onset of symptoms, whereas the survivors were treated 7 to 15 days after onset, suggesting that these patients might have been convalescing when treated. Passive transfer of neutralising antibodies protected guinea pigs (Refs 58, 68) and hymadryad baboons (Refs 66, 68, 108), and partially protected mice (Ref. 58), from EBOV infection. In contrast, passive therapy of EBOV-infected cynomolgus monkeys with a commercially available IgG from horses (hyperimmune to EBOV) delayed onset of viraemia and clinical signs but was not protective, even though the equine product had a high log neutralisation index (Refs 58, 59). Successful treatment of guinea pigs and hymadryad baboons could relate to lower viraemias and infectious viral burdens compared with those in monkeys. Some protective murine monoclonal antibodies were effective therapeutically when administered to mice after exposure to ZEBOV (Ref. 109). Also, recombinant monoclonal antibodies have been produced from convalescent human bone marrow and peripheral blood mononuclear cells (Ref. 110), and one of these recombinant human antibodies protected guinea pigs from lethal ZEBOV infection (Ref. 111). However, recent testing of this antibody in monkeys showed that the administration of the reagent delayed onset of viraemia and clinical signs but did not protect the animals from lethal ZEBOV disease (Ref. 112).

Inhibition of membrane fusion

Each stage of the viral entry process offers a potential target for interrupting viral replication. Inhibition of fusion as an antiviral modality has recently gained interest primarily based on the US Food and Drug Administration's approval of a novel fusion inhibitor (T-20, Enfuvirtide, Fuseon) for antiretroviral therapy in humans (reviewed in Ref. 113); Enfuvirtide is a peptide that mimics a critical region within the viral envelope glycoprotein gp41, thereby blocking gp41 structural rearrangements at a transitional prefusion conformation. The use of fusion

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

Table 2. Summary of treatment options for Ebola virus haemorrhagic fever

Treatment option	Feasibility and success
Antiviral approaches	
Antibody therapy	Passive immunisation using approaches including recombinant monoclonal antibodies and a polyclonal equine IgG consistently protected rodents from lethal ZEBOV HF but failed to protect nonhuman primates. Technological advances to raise circulating antibody concentrations might improve success in primates.
Fusion inhibitors	Some inhibition in vitro but approach has not been tested in vivo.
Transcription/replication inhibitors	
Nucleoside analogues	Ribavirin failed to protect guinea pigs and nonhuman primates from lethal ZEBOV HF.
Antisense oligonucleotides	In vitro studies have been inconclusive. Advances in chemistry and formulation of cell-penetrating peptides would enhance in vivo opportunities.
Small interfering RNAs	Not reported. Potential advantages over antisense oligonucleotides in efficiency and duration of effect. Specificity with minimal adverse effects is strength of technology; drawbacks include genetic variation in viruses and undeveloped delivery systems. Not yet proven in vivo.
Maturation inhibitors	
Furin inhibitors	In vitro data sparse but not encouraging. More studies needed to evaluate potential. Approval for HIV therapy in humans shows in vivo feasibility.
Budding inhibitors	In vitro data sparse but not encouraging. More studies needed.
Modulation of the host immune response	
Inflammatory response modulators	
Type I IFNs	Treatment encouraging in rodents, but failed to protect nonhuman primates from lethal ZEBOV HF. Improvement of formulations and selective use of IFN-α subtypes with greater antiviral properties might have more utility.
SAH inhibitors	Adenosine analogues, but efficacy in mice linked to induction of type I IFN response. Failed to protect nonhuman primates from lethal ZEBOV HF.
Anticytokine therapies	Abnormal production of cytokines/chemokines is a notable feature of disease in EBOV-infected primates. Anticytokine therapies used for several human diseases and conditions present opportunities.
Inhibitors of lymphocyte apoptosis	Problem will be specifically targeting approach to protect lymphocytes without concurrently enhancing survival of EBOV-infected cells.
Coagulation modulators	
Tissue factor pathway inhibitors	Pilot study in small cohort of rhesus monkeys showed partial protection, and delayed death in unprotected animals. Might have greatest utility in combinatorial approaches and/or in supportive care.
Factor X inhibitors	Blocking common pathway might be more beneficial than inhibiting a specific coagulation pathway. Success in treating deep-vein thrombosis with factor X inhibitors in humans shows potential for mitigating effects of EBOV HF.
Activated protein C	Reduced levels of plasma protein C in ZEBOV-infected nonhuman primates mirrors reduced levels associated with human sepsis. Success in treating human sepsis with a recombinant product indicates possible utility for EBOV HF.
Therapeutic vaccines	Vesicular stomatitis virus-based ZEBOV vaccine showed promising results when administered therapeutically to mice. Confirmatory studies in nonhuman primates are critically needed to determine utility for future development.
Combination therapies	Difficulty in protecting nonhuman primates using a variety of approaches suggests that improved survival might be realised by combining strategies that suppress viral replication with strategies that modulate inflammation and coagulation, allowing the host time to mount an effective immune response.

Abbreviations: EBOV, Ebola virus; IFN, interferon; HF, haemorrhagic fever; HIV, human immunodeficiency virus; ZEBOV, Zaire EBOV.

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

inhibitors against EBOV has not been thoroughly explored. Watanabe and colleagues recently demonstrated a model for EBOV GP_2-mediated membrane fusion using pseudotyped viruses (Ref. 114). This model showed the importance of a coiled-coil motif of GP_2 in mediating membrane fusion. Notably, an oligopeptide corresponding to the coiled-coil structure of GP_2 competitively inhibited EBOV entry. However, others have noted that the concentration required to attain 50% inhibition (>1 mg/ml) would be difficult to achieve in sera of test animals (Ref. 115).

Inhibition of transcription and replication

With the exception of retroviruses, all RNA viruses including EBOV encode an RNA-dependent RNA polymerase to catalyse the synthesis of new genomes and mRNAs. Thus, these viral replication enzymes (L polymerase for filoviruses) are primary targets for antiviral therapeutics. The nucleoside analogue ribavirin is a broad-spectrum antiviral drug that can block the RNA polymerase of many RNA viruses; however, ribavirin does not effectively inhibit the replication of EBOV. Notably, ribavirin does not protect guinea pigs from lethal infection with EBOV nor did it protect monkeys from lethal EBOV infection (Ref. 116). A similar compound, ribamidyl, was unable to protect hamadryad baboons against EBOV (Ref. 108). Other tactics to interfere with transcription and replication include using antisense oligonucleotides corresponding to sequences in the viral genomic RNA or mRNA. Preliminary studies employing this strategy to inhibit EBOV replication were mostly unsuccessful (Ref. 115). However, recent studies by Hartlieb et al. showed that the oligomerisation of EBOV VP30 could be dose-dependently inhibited by a synthetic peptide derived from the presumed oligomerisation domain, suggesting that VP30 oligomerisation might be a potential target for antiviral drugs (Ref. 117).

Interference with viral assembly, maturation, and budding

Unlike human immunodeficiency virus (HIV), where pharmacological blockade of a viral protease was shown to have therapeutic efficacy, EBOV does not encode its own protease that could serve as a target for an inhibitor. In fact, there is a single proteolytic step described in EBOV replication, involving the cleavage of GP_0 into GP_1 and GP_2 by a cellular furin-like protease (Refs 17, 18). In an important study, Neumann and colleagues showed that a genetically engineered ZEBOV lacking the cleavage site was able to replicate normally in vitro (Ref. 118), suggesting that even if an inhibitor could block this step in maturation, it would likely have a negligible effect on viral replication. Although replication of the ZEBOV cleavage-site mutant was approximately 1.0 log10 pfu/ml lower than wild-type ZEBOV in the same in vitro assay system (Ref. 118), ZEBOV infections in vivo are typically associated with very high viraemias, usually in excess of 7.0 log10 pfu/ml (Ref. 61). Thus, this apparent mild attenuation of the ZEBOV cleavage-site mutant would likely have little significance in vivo. However, inhibition of cleavage might have other effects on pathogenesis that are not associated with replication, and the potential role of furin inhibitors cannot be discounted (see Ref. 119 for a detailed review of furin inhibitors). Specifically, Dolnik et al. recently showed that TNF-α-converting enzyme (TACE), a member of the ADAM family of zinc-dependent metalloproteinases, is involved in EBOV GP shedding, which might play an important role in the pathogenesis of infection by efficiently blocking the activity of virus-neutralising antibodies (Ref. 120). Because furin is the major proprotein convertase involved in the maturation/activation of TACE (Ref. 121), inhibition of furin could potentially interfere with TACE and thereby mitigate the purported deleterious consequences of GP shedding.

Modulation of the host immune response

Regulation of the pro-inflammatory response

The other primary theme for treatment of EBOV infections has been modulation of the host immune response. This area has primarily involved efforts to boost innate immunity. Treatment with exogenous type I IFNs has been evaluated by several groups. A combination of ridostin (an IFN inducer) and reaferon (IFN-α2a) prolonged the mean time to death of ZEBOV-infected guinea pigs (Ref. 122). A recombinant B/D form of human IFN-α protected mice against a lethal challenge of ZEBOV, even when administered up to 2 days after infection (Ref. 115).

S-adenosylhomocysteine hydrolase (SAH) inhibitors have a protective effect against ZEBOV infection in mice (Refs 123, 124, 125). These adenosine analogues inhibit replication of

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

several DNA and RNA viruses, and their antiviral efficacy has been ascribed to reduced methylation of the 5' cap of viral mRNA by (guanine-7-)methyltransferase, which impedes translation of viral transcripts. However, Bray linked the protective effect of these SAH inhibitors in ZEBOV-infected mice to induction of a strong IFN-α response (Ref. 102).

As noted previously, strategies that have shown efficacy in rodents are often ineffective in nonhuman primates. Although SAH hydrolase inhibitors can effectively protect mice from lethal ZEBOV infection (Refs 123, 124, 125), studies have thus far been unable to demonstrate efficacy of these compounds in nonhuman primates (Ref. 126). Likewise, IFN-α showed little beneficial effect in cynomolgus monkeys treated with high doses of recombinant human IFN-α2b immediately after a high-dose challenge of ZEBOV (Ref. 59).

Other approaches to modulation of the host immune response include strategies to offset or moderate the physiological effects of disease. Pretreating rhesus monkeys with antioxidants, such as vitamin E, failed to confer any beneficial or protective effect to ZEBOV-infected rhesus monkeys (Ref. 64).

Regulation of the coagulation system

Two patients infected with MARV in 1975 were given vigorous supportive treatment and prophylactic heparin (Ref. 127). As both patients survived infection, heparin treatment was attempted on a single patient during the original outbreak of ZEBOV in 1976 (Ref. 2). Unfortunately, there was no favourable outcome in this case. As noted above, ZEBOV infection induces overexpression of the procoagulant TF in primate monocytes/macrophages (Ref. 63), suggesting that TF-pathway inhibition might ameliorate the effects of EBOV HF. Based on these data, it was postulated that blocking factor VIIa (FVIIa)/TF might be beneficial after EBOV infection (Ref. 65). In a preliminary study, nine ZEBOV-infected monkeys were treated with a protein that prevents blood clotting – recombinant nematode anticoagulant protein c2 (rNAPc2) – while three ZEBOV-infected monkeys were given a placebo control. Three of the nine treated animals survived, whereas all three animals that were given the placebo control died. Importantly, EBOV infection is nearly 100% fatal in monkeys and kills up to 90% of humans infected with the virus, so a 33% survival rate for one of the most virulent diseases known is significant and represents a key breakthrough. Other coagulation defects associated with EBOV infection of primates include thrombocytopaenia (Refs 13, 61), although the mechanism for this anomaly has not been defined. Pretreating rhesus monkeys with thromboxane inhibitors or prostacyclin to counteract platelet aggregation failed to confer any beneficial or protective effect to ZEBOV-infected rhesus monkeys (Ref. 64).

Research in progress and outstanding research questions

Antibody therapy

Passive transfer of neutralising antibodies protects mice, guinea pigs and hymadryad baboons from EBOV infection. However, passive immunotherapy in monkeys of the genus *Macaca* has been extremely disappointing. Recent data indicate that high levels of shed GP are present in the blood of EBOV-infected animals, suggesting that circulating GP might play an important role in disease pathogenesis by blocking the activity of EBOV-neutralising antibodies (Ref. 120). Although previous attempts were not completely successful in macaques, several new approaches warrant investigation before passive transfer of antibodies is abandoned as a potential therapy for EBOV HF. The availability of EBOV-immune monkeys, and the development of improved electrophoresis-based separation technologies, should facilitate the preparation of high-quality hyperimmune reagents. Passive therapy using homologous hyperimmune plasma should afford the opportunity to achieve much higher circulating antibody concentrations, which are likely necessary to offset the extreme viraemia observed in the nonhuman primate models of EBOV HF. Recently, there has been discussion about the role of antibodies in enhancing EBOV infection and potentially exacerbating disease (Refs 128, 129, 130). Although the significance of immunological enhancement has yet to be documented in vivo, any such demonstration would clearly require a re-evaluation of immunotherapeutic strategies.

RNA interference

Small interfering RNAs (siRNAs) are powerful, sequence-specific reagents designed to suppress the expression of genes in cultured mammalian cells through the process of RNA interference (RNAi) (Refs 131, 132). The small size of siRNAs,

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

compared with traditional antisense molecules, prevents activation of the mammalian dsRNA-inducible IFN system. These complexes recognise their single-stranded mRNA targets by matching RNA sequences, and direct the cleavage and destruction of any mRNAs that perfectly match one of the duplex siRNA strands, thereby preventing mRNA translation. Intracellular silencing complexes can be generated either by exogenously administered siRNA duplexes or by siRNAs processed from precursor short-hairpin (sh) RNAs expressed in the nucleus by vector-based systems (Refs 133, 134, 135). Various RNAi strategies have been employed to test the efficacies of siRNAs as potential therapeutic agents against RNA viruses. Whereas most studies demonstrated inhibition of either viral entry or production in vitro, recent studies documented that transgene expression can be suppressed in vivo. Specifically, the therapeutic potential of this technique was demonstrated by effectively targeting conserved regions of influenza virus genes in mice (Ref. 135). Although there have been no published studies evaluating the efficacy of RNAi as a strategy for suppressing EBOV replication either in vitro or in vivo, development and optimisation of this technology might lead to useful and effective treatments against EBOV HF. Indeed, the utility of this approach as an effective strategy to combat EBOV HF will depend on overcoming a number of obstacles, in particular the ability to develop an efficient drug delivery system. Progress is being made in this area, as evidenced by the recent work of Ge et al. illustrating the ability of polyethyleneimine (PEI), a cationic polymer, to promote the successful delivery of siRNA by intravenous administration in influenza-virus-infected mice (Ref. 135).

Budding inhibitors

EBOV budding is mediated by two proline-rich motifs – PPxY and PTAP – within VP40 (Refs 136, 137, 138, 139, 140). A full N-terminus of VP40 is required for budding to occur efficiently (Refs 137, 139). Optimal budding is facilitated by interaction with the cellular ubiquinating enzymes Nedd4 and Tsg101 (Refs 138, 139, 140). Thus, drugs that interfere with these interactions are of obvious interest. However, identifying compounds that have therapeutic utility will be challenging, because it is likely that concentrations required to attain sufficient inhibition would be difficult to realise in sera of test animals. Furthermore, the utility of budding inhibitors as potential therapies is called into question by recent work reported by Ebihara and colleagues (Ref. 141). Specifically, rescue of ZEBOVs containing mutations in the late domains of VP40 resulted in viral yields that were only 1.5 log10 pfu/ml less than wild-type ZEBOV; it is unlikely that such reduction would have much therapeutic benefit, considering the extremely high viral loads attained during in vivo ZEBOV infections (Ref. 61).

Immune modulators

IFNs

The use of IFN-α and inducers of type I IFNs as a therapy for treating EBOV infection has been largely unsuccessful thus far. However, an increased understanding of EBOV pathogenesis concomitant with recent advances in the production of alternative IFN therapeutic preparations might offer improved efficacy. For example, the EBOV VP35 protein reportedly prevents EBOV-induced transcription of IFN-β (Ref. 101). Moreover, induction of IFN-β appears to be a late event in disease pathogenesis of ZEBOV-infected nonhuman primates (Ref. 61). Therefore, therapeutic treatment of animals with recombinant IFN-β might successfully reduce EBOV viraemia and thus ameliorate the effects of lethal disease. Other potential therapies include the use of universal type I IFNs and other IFN formulations that have increased bioavailability.

Anticytokine interventions

There have been relatively few attempts to modulate the dysregulated cytokine/chemokine response that is a consistent feature among many of the viral HFs. Treating MARV-infected guinea pigs with desferal, an immunomodulator that is an IL-1 and TNF-α antagonist, partially protected a small cohort of animals (Ref. 142). In another study, treating MARV-infected guinea pigs with IL-1 receptor antagonist (IL-1RA) or anti-TNF-α serum administered on days 4 to 7 after infection decreased the concentration of circulating TNF-α and protected half of the animals from lethal infection (Ref. 143). Anti-TNF therapy also reduced mortality in a lethal mouse model of dengue virus infection (Ref. 144). Increased plasma levels of TNF-α have been shown in ZEBOV-infected humans and nonhuman primates (Refs 61, 77, 96). It would be interesting to determine whether anti-TNF therapy has any beneficial effect in lethal animal models of EBOV

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

HF. Pro-inflammatory cytokines such as IL-6 have been shown to effectively upregulate the procoagulant TF expression on monocytes (Refs 93, 145). Elevations of plasma IL-6 have been reported by day 4 postinfection in ZEBOV-infected monkeys (Ref. 61). Given the potentially important pathogenic role of IL-6 in EBOV HF, the in vivo neutralisation of IL-6 could have therapeutic utility in ameliorating the lethal effects of EBOV HF. Anti-IL-6 approaches can mitigate the deleterious effects of endotoxin-induced sepsis in several animal models (Refs 146, 147, 148). Additional benefits might be realised by using drugs that target NF-κB activity because many of these cytokines/chemokines can be upregulated by NF-κB.

Inhibition of apoptosis of bystander lymphocytes

It is likely that the marked apoptosis of bystander lymphocytes contributes to the immunosuppression that characterises EBOV infection of primates. Therefore, successful inhibition of bystander lymphocyte apoptosis offers an attractive therapeutic target for mitigating the lethal effects of EBOV HF. The exact mechanism(s) for triggering apoptosis pathways activated during EBOV infection of primates remains unknown. Although it will be important to determine whether EBOV proteins exhibit pro-apoptotic activities, it is clear that increased expression of pro-apoptotic genes such as TRAIL and Fas during EBOV infections are contributing factors to the observed lymphocyte apoptosis and thus are obvious targets for intervention strategies. Therapies directed to inhibit Fas and/or TRAIL function might have a beneficial effect. These therapeutic interventions are currently being tested in other disease models. For example, treatment using a neutralising anti-TRAIL monoclonal antibody in HIV-infected mice significantly reduced the development of apoptotic $CD4^+$ T cells (Ref. 149). Caspase inhibitors have also shown some benefit in murine models of sepsis (Ref. 150). However, the targeted blockade of apoptosis to lymphocytes, and not to cell populations that harbour EBOV, is a formidable hurdle that must be overcome before this therapeutic strategy can be fully realised.

Coagulation modulators

Dysregulation of the coagulation system appears to be important to the development of haemorrhagic shock and death in fatal cases of ZEBOV. The first therapeutic treatment for EBOV HF was recently demonstrated in nonhuman primates (Ref. 65). This treatment regimen was based on targeting the mechanism by which the coagulation cascade was activated during EBOV HF. In particular, identifying the importance of the TF pathway provided a rational approach to identify and utilise rNAPc2 in an attempt to mitigate the severe effects of EBOV infection. As rNAPc2 primarily targets signalling through the extrinsic blood coagulation pathway, additional benefits might be realised by using inhibitors of FX to target the intrinsic pathway as well.

In other recent studies, we showed rapid drops in levels of plasma protein C in ZEBOV-infected nonhuman primates, with the initial decrease observed as early as the first day after ZEBOV challenge (Ref. 63). This work suggests that protein C might be a critical component to the observed coagulation dysfunction in ZEBOV HF. The protein C system is one of the main anticoagulant mechanisms in blood (Ref. 151). Growing evidence also suggests that protein C has direct anti-inflammatory properties and modulating activity on cellular functions, likely by blocking NF-κB nuclear translocation (Ref. 152). Reduced levels of protein C are found in the majority of patients with sepsis and are associated with an increased risk of death (Ref. 153). From a therapeutic perspective, treatment with recombinant human activated protein C significantly reduced mortality in patients with severe sepsis (Ref. 154). Clearly, identifying protein C abnormalities in primate models of EBOV HF (which so closely parallel protein C anomalies in human coagulopathies) offers an ideal target for chemotherapeutic interventions.

Therapeutic vaccines

Recently, significant progress was made towards the development of an EBOV vaccine. Thus far, three EBOV vaccine platforms have been tested successfully in nonhuman primates: (1) a prime–boost strategy consisting of a DNA vaccine prime followed by an adenovirus (ADV) vaccine boost (Ref. 60); (2) an accelerated ADV vaccine consisting of a single injection of ADV expressing EBOV proteins (Ref. 45); and (3), most recently, a recombinant vesicular stomatitis virus (rVSV)-based vaccine (Ref. 155). All of these candidate vaccines are based on ZEBOV and have shown

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

protective efficacy against homologous ZEBOV challenge, but it is unknown whether the ZEBOV proteins incorporated into these vaccines will also confer protection against other species of EBOV. Indeed, few studies have challenged animals immune to one species of EBOV with a different species of EBOV. Small cohorts of nonhuman primates immune to either REBOV or SEBOV were only partially protected from lethal ZEBOV challenge (Refs 55, 70), suggesting that multivalent vaccine approaches might be necessary.

Postexposure vaccination has utility in preventing or modifying disease for a number of viral agents including rabies (reviewed in Ref. 156), hepatitis B (Ref. 157), and smallpox (Ref. 158). Recently, a ZEBOV vaccine based on rVSV protected eight of ten mice when therapeutically administered 30 min after receiving a lethal dose (>1000 LD50) of ZEBOV (Ref. 155). The mechanism of protection has not been determined. It is worth noting the studies of Baize and colleagues on the immune response in fatalities and survivors during two outbreaks of ZEBOV HF in Gabon (Ref. 77). Their results suggest that recovery from EBOV HF is associated with early and robust humoral responses. Furthermore, cytotoxic cell activation was noted among PBMCs of recovering patients at the end of the disease. The same investigators subsequently reported that inflammatory responses are critical determinants in innate and adaptive immune responses (Ref. 159). They noted that the rapid release of pro-inflammatory cytokines in asymptomatic ZEBOV infections suggests that this response might be involved in controlling viral replication and inducing specific immunity. If the outcome of EBOV infection is mostly determined by host responses during the early stages of the incubation period, then interventions to augment those defences, such as therapeutic vaccines, might help to prevent or ameliorate the disease.

Concluding remarks

There is a critical need for the development of effective therapies to respond to outbreaks of EBOV in Africa and to counter potential acts of bioterrorism. In addition, a potential EBOV exposure involving a researcher at a US Army laboratory (Ref. 160), and the recent death of a Russian scientist after an accidental exposure to EBOV (Ref. 161), emphasise the need for medical countermeasures for postexposure prophylaxis. Considering the aggressive nature of EBOV infections, in particular the rapid and overwhelming viral burdens, early diagnosis will play a significant role in determining the success of any intervention strategy. Thus far, antiviral modalities targeting EBOV replication have largely been unsuccessful. Strategies showing some promise in rodents have uniformly failed to protect nonhuman primates from lethal EBOV HF (Refs 58, 59, 111, 112, 116, 123, 124, 125, 126). Currently, among the most promising antiviral approaches are siRNAs. Assessment of this approach in in vivo models hinges on the ability to develop an efficient drug delivery system, and an intense effort is under way towards achieving this goal.

Results of in vivo studies performed to date suggest that there might be no single treatment to combat the lethal effects of EBOV HF. Successful treatment might require combining several beneficial approaches, such as strategies targeting viral replication with modalities controlling the manifestations of disease. A beneficial effect using an inhibitor, rNAPc2, of the TF pathway has been observed (Ref. 65). It will be important to determine whether survival can be improved by combining coagulation-modulating agents such as rNAPc2 with agents that have antiviral properties. Moreover, it will be important to evaluate whether recombinant activated protein C provides synergistic benefit with other coagulation-modulating agents, such as rNAPc2. Therapeutic vaccines might also have utility in combination approaches. In some cases, therapeutic vaccines are administered in conjunction with other treatments including immune globulin to improve efficacy (reviewed in Ref. 156). It will be interesting to determine whether the therapeutic efficacy of rNAPc2 can be improved by employing this anticoagulant protein in combination with therapeutic vaccines. Clearly, an increased understanding of the mechanisms of EBOV pathogenesis will facilitate our ability to develop effective countermeasures against this notoriously aggressive pathogen.

Acknowledgements and funding

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army. We thank the peer reviewers of this article for their comments. The authors' work on Ebola and Marburg viruses was supported by the Medical

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

Chemical/Biological Defense Research Program, US Army Medical Research and Material Command (project numbers 02-4-4J-081, 02-4-4J-084, 03-4-7J-020, and 04-4-7J-012).

References

1 Khan, A.S. et al. (1999) The reemergence of Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis 179 Suppl 1, S76-86, PubMed: 9988168
2 Isaacson, M. et al. (1978) Clinical aspects of Ebola virus disease at the Ngaliema hospital, Kinshasa, Zaire, 1976. In Ebola Virus Haemorrhagic Fever (Pattyn, S.R., ed.), pp. 15-20, Elsevier/North-Holland Biomedical Press, New York
3 Piot, P. et al. (1978) Clinical aspects of Ebola virus infection in Yambuku area, Zaire, 1976. In Ebola Virus Haemorrhagic Fever (Pattyn, S.R., ed.), pp. 7-14, Elsevier/North-Holland Biomedical Press, New York
4 Smith, D.H., Francis, F. and Simpson, D.I.H. (1978) African haemorrhagic fever in the southern Sudan, 1976: the clinical manifestations. In Ebola Virus Haemorrhagic Fever (Pattyn, S.R., ed.), pp. 21-26, Elsevier/North-Holland Biomedical Press, New York
5 (1978) Ebola haemorrhagic fever in Sudan, 1976. Report of a WHO/International Study Team. Bull World Health Organ 56, 247-270, PubMed: 307455
6 (1978) Ebola haemorrhagic fever in Zaire, 1976. Bull World Health Organ 56, 271-293, PubMed: 307456
7 Bwaka, M.A. et al. (1999) Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. J Infect Dis 179 Suppl 1, S1-7, PubMed: 9988155
8 Leroy, E.M. et al. (2001) Early immune responses accompanying human asymptomatic Ebola infections. Clin Exp Immunol 124, 453-460, PubMed: 11472407
9 Georges-Courbot, M.C. et al. (1997) Isolation and phylogenetic characterization of Ebola viruses causing different outbreaks in Gabon. Emerg Infect Dis 3, 59-62, PubMed: 9126445
10 Walsh, P.D. et al. (2003) Catastrophic ape decline in western equatorial Africa. Nature 422, 611-614, PubMed: 12679788
11 Jahrling, P.B. et al. (1990) Preliminary report: isolation of Ebola virus from monkeys imported to USA. Lancet 335, 502-505, PubMed: 1968529
12 Le Guenno, B. et al. (1995) Isolation and partial characterisation of a new strain of Ebola virus. Lancet 345, 1271-1274, PubMed: 7746057
13 Sanchez, A. et al. (2001) Marburg and Ebola viruses. In Fields Virology (Knipe, D.M. and Howley, P.M., eds), pp. 1279-1304, Lippincott Williams & Wilkins, Philadelphia
14 Sanchez, A. et al. (1993) Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. Virus Res 29, 215-240, PubMed: 8237108
15 Takada, A. et al. (1997) A system for functional analysis of Ebola virus glycoprotein. Proc Natl Acad Sci U S A 94, 14764-14769, PubMed: 9405687
16 Ito, H. et al. (1999) Mutational analysis of the putative fusion domain of Ebola virus glycoprotein. J Virol 73, 8907-8912, PubMed: 10482652
17 Sanchez, A. et al. (1998) Biochemical analysis of the secreted and virion glycoproteins of Ebola virus. J Virol 72, 6442-6447, PubMed: 9658086
18 Volchkov, V.E. et al. (1998) Processing of the Ebola virus glycoprotein by the proprotein convertase furin. Proc Natl Acad Sci U S A 95, 5762-5767, PubMed: 9576958
19 Volchkov, V.E. et al. (1995) GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. Virology 214, 421-430, PubMed: 8553543
20 Sanchez, A. et al. (1996) The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proc Natl Acad Sci U S A 93, 3602-3607, PubMed: 8622982
21 Volchkov, V.E. et al. (2001) Recovery of infectious Ebola virus from complementary DNA: RNA editing of the GP gene and viral cytotoxicity. Science 291, 1965-1969, PubMed: 11239157
22 Noda, T. et al. (2002) Ebola virus VP40 drives the formation of virus-like filamentous particles along with GP. J Virol 76, 4855-4865, PubMed: 11967302
23 Huang, Y. et al. (2002) The assembly of Ebola virus nucleocapsid requires virion-associated proteins 35 and 24 and posttranslational modification of nucleoprotein. Mol Cell 10, 307-316, PubMed: 12191476
24 Han, Z. et al. (2003) Biochemical and functional characterization of the Ebola virus VP24 protein: implications for a role in virus assembly and budding. J Virol 77, 1793-1800, PubMed: 12525613
25 Watanabe, S. et al. (2004) Production of novel

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

ebola virus-like particles from cDNAs: an alternative to ebola virus generation by reverse genetics. J Virol 78, 999-1005, PubMed: 14694131
26 Geisbert, T.W. and Jahrling, P.B. (1995) Differentiation of filoviruses by electron microscopy. Virus Res 39, 129-150, PubMed: 8837880
27 Empig, C.J. and Goldsmith, M.A. (2002) Association of the caveola vesicular system with cellular entry by filoviruses. J Virol 76, 5266-5270, PubMed: 11967340
28 Simmons, G. et al. (2003) Folate receptor alpha and caveolae are not required for Ebola virus glycoprotein-mediated viral infection. J Virol 77, 13433-13438, PubMed: 14645601
29 Takada, A. et al. (2000) Downregulation of beta1 integrins by Ebola virus glycoprotein: implication for virus entry. Virology 278, 20-26, PubMed: 11112476
30 Chan, S.Y. et al. (2001) Folate receptor-alpha is a cofactor for cellular entry by Marburg and Ebola viruses. Cell 106, 117-126, PubMed: 11461707
31 Alvarez, C.P. et al. (2002) C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J Virol 76, 6841-6844, PubMed: 12050398
32 Lin, G. et al. (2003) Differential N-linked glycosylation of human immunodeficiency virus and Ebola virus envelope glycoproteins modulates interactions with DC-SIGN and DC-SIGNR. J Virol 77, 1337-1346, PubMed: 12502850
33 Simmons, G. et al. (2003) DC-SIGN and DC-SIGNR bind ebola glycoproteins and enhance infection of macrophages and endothelial cells. Virology 305, 115-123, PubMed: 12504546
34 Takada, A. et al. (2004) Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. J Virol 78, 2943-2947, PubMed: 14990712
35 Sinn, P.L. et al. (2003) Lentivirus vectors pseudotyped with filoviral envelope glycoproteins transduce airway epithelia from the apical surface independently of folate receptor alpha. J Virol 77, 5902-5910, PubMed: 12719583
36 Baskerville, A. et al. (1985) Ultrastructural pathology of experimental Ebola haemorrhagic fever virus infection. J Pathol 147, 199-209, PubMed: 4067737
37 Geisbert, T.W. et al. (1992) Association of Ebola-related Reston virus particles and antigen with tissue lesions of monkeys imported to the United States. J Comp Pathol 106, 137-152, PubMed: 1597531
38 Jaax, N.K. et al. (1996) Lethal experimental infection of rhesus monkeys with Ebola-Zaire (Mayinga) virus by the oral and conjunctival route of exposure. Arch Pathol Lab Med 120, 140-155, PubMed: 8712894
39 Davis, K.J. et al. (1997) Pathology of experimental Ebola virus infection in African green monkeys. Involvement of fibroblastic reticular cells. Arch Pathol Lab Med 121, 805-819, PubMed: 9278608
40 Zaki, S.R. and Goldsmith, C.S. (1999) Pathologic features of filovirus infections in humans. Curr Top Microbiol Immunol 235, 97-116, PubMed: 9893381
41 Muhlberger, E. (2004) Genome organization, replication, and transcription of filoviruses. In Ebola and Marburg Viruses: Molecular and Cellular Biology (Klenk, H.D. and Feldmann, H., eds), pp. 1-26, Horizon Bioscience, Norfolk, UK
42 Geisbert, T.W. et al. (2004) Filovirus pathogenesis in nonhuman primates. In Ebola and Marburg Viruses: Molecular and Cellular Biology (Klenk, H.D. and Feldmann, H., eds), pp. 203-238, Horizon Bioscience, Norfolk, UK
43 Bray, M. (2004) Pathogenesis of filovirus infection in mice. In Ebola and Marburg Viruses: Molecular and Cellular Biology (Klenk, H.D. and Feldmann, H., eds), pp. 255-277, Horizon Bioscience, Norfolk, UK
44 Geisbert, T.W. et al. (2002) Evaluation in nonhuman primates of vaccines against Ebola virus. Emerg Infect Dis 8, 503-507, PubMed: 11996686
45 Sullivan, N.J. et al. (2003) Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. Nature 424, 681-684, PubMed: 12904795
46 Breman, J.G. et al. (1978) The epidemiology of Ebola hemorrhagic fever in Zaire, 1976. In Ebola Virus Haemorrhagic Fever (Pattyn, S.R., ed.), pp. 103-121, Elsevier/North-Holland Biomedical Press, New York
47 Ryabchikova, E. et al. (1996) Ebola virus infection in guinea pigs: presumable role of granulomatous inflammation in pathogenesis. Arch Virol 141, 909-921, PubMed: 8678836
48 Chepurnov, A.A. et al. (1997) [Change in biochemical and hemostatic indicators in guinea pigs upon administering Ebola virus preparations]. Vopr Virusol 42, 171-175, PubMed: 9304298
49 Bray, M. et al. (1998) A mouse model for evaluation of prophylaxis and therapy of Ebola

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

hemorrhagic fever. J Infect Dis 178, 651-661, PubMed: 9728532
50 Connolly, B.M. et al. (1999) Pathogenesis of experimental Ebola virus infection in guinea pigs. J Infect Dis 179 Suppl 1, S203-217, PubMed: 9988186
51 Bray, M. et al. (2001) Haematological, biochemical and coagulation changes in mice, guinea-pigs and monkeys infected with a mouse-adapted variant of Ebola Zaire virus. J Comp Pathol 125, 243-253, PubMed: 11798241
52 Geisbert, T.W. et al. (2000) Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. Lab Invest 80, 171-186, PubMed: 10701687
53 Gibb, T.R. et al. (2001) Pathogenesis of experimental Ebola Zaire virus infection in BALB/c mice. J Comp Pathol 125, 233-242, PubMed: 11798240
54 Bowen, E.T. et al. (1978) Ebola haemorrhagic fever: experimental infection of monkeys. Trans R Soc Trop Med Hyg 72, 188-191, PubMed: 418537
55 Fisher-Hoch, S.P. et al. (1992) Pathogenic potential of filoviruses: role of geographic origin of primate host and virus strain. J Infect Dis 166, 753-763, PubMed: 1527410
56 Ryabchikova, E.I., Kolesnikova, L.V. and Luchko, S.V. (1999) An analysis of features of pathogenesis in two animal models of Ebola virus infection. J Infect Dis 179 Suppl 1, S199-202, PubMed: 9988185
57 Ryabchikova, E.I., Kolesnikova, L.V. and Netesov, S.V. (1999) Animal pathology of filoviral infections. Curr Top Microbiol Immunol 235, 145-173, PubMed: 9893383
58 Jahrling, P.B. et al. (1996) Passive immunization of Ebola virus-infected cynomolgus monkeys with immunoglobulin from hyperimmune horses. Arch Virol Suppl 11, 135-140, PubMed: 8800795
59 Jahrling, P.B. et al. (1999) Evaluation of immune globulin and recombinant interferon-alpha2b for treatment of experimental Ebola virus infections. J Infect Dis 179 Suppl 1, S224-234, PubMed: 9988188
60 Sullivan, N.J. et al. (2000) Development of a preventive vaccine for Ebola virus infection in primates. Nature 408, 605-609, PubMed: 11117750
61 Geisbert, T.W. et al. (2003) Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: evidence that dendritic cells are early and sustained targets of infection. Am J Pathol 163, 2347-2370, PubMed: 14633608
62 Geisbert, T.W. et al. (2003) Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. Am J Pathol 163, 2371-2382, PubMed: 14633609
63 Geisbert, T.W. et al. (2003) Mechanisms underlying coagulation abnormalities in ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. J Infect Dis 188, 1618-1629, PubMed: 14639531
64 Fisher-Hoch, S.P. et al. (1985) Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). J Infect Dis 152, 887-894, PubMed: 4045253
65 Geisbert, T.W. et al. (2003) Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. Lancet 362, 1953-1958, PubMed: 14683653
66 Mikhailov, V.V. et al. (1994) [The evaluation in hamadryas baboons of the possibility for the specific prevention of Ebola fever]. Vopr Virusol 39, 82-84, PubMed: 8017061
67 Chepurnov, A.A. et al. (1995) Attempts to obtain a vaccine against Ebola fever. Vopr Virusol 40, 257-260, PubMed: 8686261
68 Kudoyarova-Zubavichene, N.M. et al. (1999) Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. J Infect Dis 179 Suppl 1, S218-223, PubMed: 9988187
69 Ellis, D.S. et al. (1978) Ebola virus: a comparison, at ultrastructural level, of the behaviour of the Sudan and Zaire strains in monkeys. Br J Exp Pathol 59, 584-593, PubMed: 106868
70 Bowen, E.T. et al. (1980) A comparative study of strains of Ebola virus isolated from southern Sudan and northern Zaire in 1976. J Med Virol 6, 129-138, PubMed: 6165800
71 Jahrling, P.B. et al. (1996) Experimental infection of cynomolgus macaques with Ebola-Reston filoviruses from the 1989-1990 U.S. epizootic. Arch Virol Suppl 11, 115-134, PubMed: 8800793
72 Formenty, P. et al. (1999) Ebola virus outbreak among wild chimpanzees living in a rain forest of Cote d'Ivoire. J Infect Dis 179 Suppl 1, S120-126, PubMed: 9988175
73 Schnittler, H.J. and Feldmann, H. (1998) Marburg and Ebola hemorrhagic fevers: does the primary course of infection depend on the accessibility of organ-specific macrophages? Clin Infect Dis 27,

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

404-406, PubMed: 9709901
74 Stroher, U. et al. (2001) Infection and activation of monocytes by Marburg and Ebola viruses. J Virol 75, 11025-11033, PubMed: 11602743
75 Baumann, H. and Gauldie, J. (1994) The acute phase response. Immunol Today 15, 74-80, PubMed: 7512342
76 Hensley, L.E. et al. (2002) Proinflammatory response during Ebola virus infection of primate models: possible involvement of the tumor necrosis factor receptor superfamily. Immunol Lett 80, 169-179, PubMed: 11803049
77 Baize, S. et al. (1999) Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat Med 5, 423-426, PubMed: 10202932
78 Reed, D.S. et al. (2004) Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic fever in cynomolgus macaques. Viral Immunol 17, 390-400, PubMed: 15357905
79 Mahanty, S. et al. (2003) Cutting edge: impairment of dendritic cells and adaptive immunity by Ebola and Lassa viruses. J Immunol 170, 2797-2801, PubMed: 12626527
80 Daniel, P.T. et al. (1998) Dendritic cells prevent CD95 mediated T lymphocyte death through costimulatory signals. Adv Exp Med Biol 451, 173-177, PubMed: 10026869
81 Yang, Z. et al. (1998) Distinct cellular interactions of secreted and transmembrane Ebola virus glycoproteins. Science 279, 1034-1037, PubMed: 9461435
82 Yang, Z.Y. et al. (2000) Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. Nat Med 6, 886-889, PubMed: 10932225
83 Dvorak, H.F. et al. (1995) Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 146, 1029-1039, PubMed: 7538264
84 Kevil, C.G. et al. (1998) Vascular permeability factor/vascular endothelial cell growth factor-mediated permeability occurs through disorganization of endothelial junctional proteins. J Biol Chem 273, 15099-15103, PubMed: 9614120
85 Walker, D.H. (1988) Pathology and pathogenesis of the vasculotropic rickettsioses. In Biology of Rickettsial Diseases (Walker, D.H., ed.), pp. 115-138, CRC Press, Boca Raton
86 Wong, K.T. et al. (2002) Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 161, 2153-2167, PubMed: 12466131
87 Murphy, F.A. (1978) Pathology of Ebola virus infection. In Ebola Virus Haemorrhagic Fever (Pattyn, S.R., ed.), pp. 43-60, Elsevier/North-Holland Biomedical Press, New York
88 Chan, S.Y., Ma, M.C. and Goldsmith, M.A. (2000) Differential induction of cellular detachment by envelope glycoproteins of Marburg and Ebola (Zaire) viruses. J Gen Virol 81, 2155-2159, PubMed: 10950971
89 Simmons, G. et al. (2002) Ebola virus glycoproteins induce global surface protein down-modulation and loss of cell adherence. J Virol 76, 2518-2528, PubMed: 11836430
90 Calandra, T. et al. (1991) High circulating levels of interleukin-6 in patients with septic shock: evolution during sepsis, prognostic value, and interplay with other cytokines. The Swiss-Dutch J5 Immunoglobulin Study Group. Am J Med 91, 23-29, PubMed: 1907101
91 Damas, P. et al. (1992) Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. Ann Surg 215, 356-362, PubMed: 1558416
92 Marty, C. et al. (1994) Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. Crit Care Med 22, 673-679, PubMed: 8143477
93 Grignani, G. and Maiolo, A. (2000) Cytokines and hemostasis. Haematologica 85, 967-972, PubMed: 10980636
94 Slifka, M.K. and Whitton, J.L. (2000) Clinical implications of dysregulated cytokine production. J Mol Med 78, 74-80, PubMed: 10794542
95 Cohen, J. (2002) The immunopathogenesis of sepsis. Nature 420, 885-891, PubMed: 12490963
96 Villinger, F. et al. (1999) Markedly elevated levels of interferon (IFN)-gamma, IFN-alpha, interleukin (IL)-2, IL-10, and tumor necrosis factor-alpha associated with fatal Ebola virus infection. J Infect Dis 179 Suppl 1, S188-191, PubMed: 9988183
97 Hutchinson, K.L. et al. (2001) Multiplex analysis of cytokines in the blood of cynomolgus macaques naturally infected with Ebola virus (Reston serotype). J Med Virol 65, 561-566, PubMed: 11596094
98 Dalgard, D.W. et al. (1992) Combined simian hemorrhagic fever and Ebola virus infection in

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

cynomolgus monkeys. Lab Anim Sci 42, 152-157, PubMed: 1318446
99 Basler, C.F. et al. (2000) The Ebola virus VP35 protein functions as a type I IFN antagonist. Proc Natl Acad Sci U S A 97, 12289-12294, PubMed: 11027311
100 Basler, C.F. et al. (2003) The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. J Virol 77, 7945-7956, PubMed: 12829834
101 Basler, C.F. and Palese, P. (2004) Modulation of innate immunity by filoviruses. In Ebola and Marburg Viruses: Molecular and Cellular Biology (Klenk, H.D. and Feldmann, H., eds), pp. 305-349, Horizon Bioscience, Norfolk, UK
102 Bray, M. (2001) The role of the Type I interferon response in the resistance of mice to filovirus infection. J Gen Virol 82, 1365-1373, PubMed: 11369881
103 Mammen, E.F. (2000) Disseminated intravascular coagulation (DIC). Clin Lab Sci 13, 239-245, PubMed: 11586511
104 Levi, M. (2004) Current understanding of disseminated intravascular coagulation. Br J Haematol 124, 567-576, PubMed: 14871243
105 Emond, R.T. et al. (1977) A case of Ebola virus infection. Br Med J 2, 541-544, PubMed: 890413
106 Mupapa, K. et al. (1999) Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. International Scientific and Technical Committee. J Infect Dis 179 Suppl 1, S18-23, PubMed: 9988160
107 Sadek, R.F. et al. (1999) Ebola hemorrhagic fever, Democratic Republic of the Congo, 1995: determinants of survival. J Infect Dis 179 Suppl 1, S24-27, PubMed: 9988161
108 Markin, V.A. et al. (1997) [Developing principles for emergency prevention and treatment of Ebola fever]. Vopr Virusol 42, 31-34, PubMed: 9103042
109 Wilson, J.A. et al. (2000) Epitopes involved in antibody-mediated protection from Ebola virus. Science 287, 1664-1666, PubMed: 10698744
110 Maruyama, T. et al. (1999) Ebola virus can be effectively neutralized by antibody produced in natural human infection. J Virol 73, 6024-6030, PubMed: 10364354
111 Parren, P.W. et al. (2002) Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. J Virol 76, 6408-6412, PubMed: 12021376
112 Parren, P.W.H.I. et al. (2003) Antibody activity against Ebola virus in vitro and in vivo. In VRC Symposium on Viral Hemorrhagic Fevers, Vaccine Research Center, NIAID, NIH, DHHS, Bethesda, MD, USA
113 Este, J.A. (2003) Virus entry as a target for anti-HIV intervention. Curr Med Chem 10, 1617-1632, PubMed: 12871111
114 Watanabe, S. et al. (2000) Functional importance of the coiled-coil of the Ebola virus glycoprotein. J Virol 74, 10194-10201, PubMed: 11024148
115 Bray, M. and Paragas, J. (2002) Experimental therapy of filovirus infections. Antiviral Res 54, 1-17, PubMed: 11888653
116 Huggins, J.W. (1989) Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad-spectrum antiviral drug. Rev Infect Dis 11 Suppl 4, S750-761, PubMed: 2546248
117 Hartlieb, B. et al. (2003) Oligomerization of Ebola virus VP30 is essential for viral transcription and can be inhibited by a synthetic peptide. J Biol Chem 278, 41830-41836, PubMed: 12912982
118 Neumann, G. et al. (2002) Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. J Virol 76, 406-410, PubMed: 11739705
119 Taylor, N.A., Van De Ven, W.J. and Creemers, J.W. (2003) Curbing activation: proprotein convertases in homeostasis and pathology. Faseb J 17, 1215-1227, PubMed: 12832286
120 Dolnik, O. et al. (2004) Ectodomain shedding of the glycoprotein GP of Ebola virus. Embo J 23, 2175-2184, PubMed: 15103332
121 Peiretti, F. et al. (2003) Intracellular maturation and transport of tumor necrosis factor alpha converting enzyme. Exp Cell Res 285, 278-285, PubMed: 12706122
122 Sergeev, A.N. et al. (1997) [Study of the treatment-prophylactic effect of immunomodulators in experimental infections, caused by Marburg, Ebola, and Venezuelan equine encephalitis viruses]. Vopr Virusol 42, 226-229, PubMed: 9424849
123 Huggins, J., Zhang, Z.X. and Bray, M. (1999) Antiviral drug therapy of filovirus infections: S-adenosylhomocysteine hydrolase inhibitors inhibit Ebola virus in vitro and in a lethal mouse model. J Infect Dis 179 Suppl 1, S240-247, PubMed: 9988190
124 Bray, M., Driscoll, J. and Huggins, J.W. (2000) Treatment of lethal Ebola virus infection in mice with a single dose of an S-adenosyl-L-homocysteine hydrolase inhibitor. Antiviral Res

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

45, 135-147, PubMed: 10809022
125 Bray, M. et al. (2002) 3-deazaneplanocin A induces massively increased interferon-alpha production in Ebola virus-infected mice. Antiviral Res 55, 151-159, PubMed: 12076759
126 Huggins, J.W. et al. (1995) Inhibition of Ebola virus by S-adenosylhomocysteine hydrolase inhibitors. Antiviral Res 26, A301
127 Gear, J.S. et al. (1975) Outbreake of Marburg virus disease in Johannesburg. Br Med J 4, 489-493, PubMed: 811315
128 Takada, A. et al. (2001) Infectivity-enhancing antibodies to Ebola virus glycoprotein. J Virol 75, 2324-2330, PubMed: 11160735
129 Geisbert, T.W. et al. (2002) Evidence against an important role for infectivity-enhancing antibodies in Ebola virus infections. Virology 293, 15-19, PubMed: 11853394
130 Takada, A. et al. (2003) Antibody-dependent enhancement of Ebola virus infection. J Virol 77, 7539-7544, PubMed: 12805454
131 Caplen, N.J. et al. (2001) Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. Proc Natl Acad Sci U S A 98, 9742-9747, PubMed: 11481446
132 Elbashir, S.M. et al. (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411, 494-498, PubMed: 11373684
133 Brummelkamp, T.R., Bernards, R. and Agami, R. (2002) A system for stable expression of short interfering RNAs in mammalian cells. Science 296, 550-553, PubMed: 11910072
134 Jacque, J.M., Triques, K. and Stevenson, M. (2002) Modulation of HIV-1 replication by RNA interference. Nature 418, 435-438, PubMed: 12087358
135 Ge, Q. et al. (2004) Inhibition of influenza virus production in virus-infected mice by RNA interference. Proc Natl Acad Sci U S A 101, 8676-8681, PubMed: 15173599
136 Harty, R.N. et al. (2000) A PPxY motif within the VP40 protein of Ebola virus interacts physically and functionally with a ubiquitin ligase: implications for filovirus budding. Proc Natl Acad Sci U S A 97, 13871-13876, PubMed: 11095724
137 Jasenosky, L.D. et al. (2001) Ebola virus VP40-induced particle formation and association with the lipid bilayer. J Virol 75, 5205-5214, PubMed: 11333902
138 Licata, J.M. et al. (2003) Overlapping motifs (PTAP and PPEY) within the Ebola virus VP40 protein function independently as late budding domains: involvement of host proteins TSG101 and VPS-4. J Virol 77, 1812-1819, PubMed: 12525615
139 Timmins, J. et al. (2003) Ebola virus matrix protein VP40 interaction with human cellular factors Tsg101 and Nedd4. J Mol Biol 326, 493-502, PubMed: 12559917
140 Yasuda, J. et al. (2003) Nedd4 regulates egress of Ebola virus-like particles from host cells. J Virol 77, 9987-9992, PubMed: 12941909
141 Ebihara, H. et al. (2003) Reverse genetics approaches to study Ebola virus. In VRC Symposium on Viral Hemorrhagic Fevers, Vaccine Research Center, NIAID, NIH, DHHS, Bethesda, MD, USA
142 Ignat'ev, G.M. et al. (1996) [Experimental study of possible treatment of Marburg hemorrhagic fever with desferal, ribavirin, and homologous interferon]. Vopr Virusol 41, 206-209, PubMed: 8967064
143 Ignatyev, G. et al. (2000) Experimental study on the possibility of treatment of some hemorrhagic fevers. J Biotechnol 83, 67-76, PubMed: 11000462
144 Atrasheuskaya, A. et al. (2003) Anti-TNF antibody treatment reduces mortality in experimental dengue virus infection. FEMS Immunol Med Microbiol 35, 33-42, PubMed: 12589955
145 Neumann, F.J. et al. (1997) Effect of human recombinant interleukin-6 and interleukin-8 on monocyte procoagulant activity. Arterioscler Thromb Vasc Biol 17, 3399-3405, PubMed: 9437185
146 Levi, M. et al. (1998) Differential effects of anti-cytokine treatment on bronchoalveolar hemostasis in endotoxemic chimpanzees. Am J Respir Crit Care Med 158, 92-98, PubMed: 9655712
147 Pallua, N., Low, J.F. and von Heimburg, D. (2003) Pathogenic role of interleukin-6 in the development of sepsis. Part II: Significance of anti-interleukin-6 and anti-soluble interleukin-6 receptor-alpha antibodies in a standardized murine contact burn model. Crit Care Med 31, 1495-1501, PubMed: 12771624
148 Riedemann, N.C. et al. (2003) Protective effects of IL-6 blockade in sepsis are linked to reduced C5a receptor expression. J Immunol 170, 503-507, PubMed: 12496437
149 Miura, Y. et al. (2001) Critical contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to apoptosis of human CD4+ T

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

cells in HIV-1-infected hu-PBL-NOD-SCID mice. J Exp Med 193, 651-660, PubMed: 11238596
150 Hotchkiss, R.S. et al. (2000) Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. Nat Immunol 1, 496-501, PubMed: 11101871
151 Esmon, C.T. (2000) Regulation of blood coagulation. Biochim Biophys Acta 1477, 349-360, PubMed: 10708869
152 Esmon, C.T. (2001) Protein C anticoagulant pathway and its role in controlling microvascular thrombosis and inflammation. Crit Care Med 29, S48-51; discussion 51-42, PubMed: 11445734
153 Lorente, J.A. et al. (1993) Time course of hemostatic abnormalities in sepsis and its relation to outcome. Chest 103, 1536-1542, PubMed: 8486040
154 Bernard, G.R. et al. (2001) Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 344, 699-709, PubMed: 11236773
155 Jones, S. et al. (2003) Replicating vectors for vaccine development. In VRC Symposium on Viral Hemorrhagic Fevers, Vaccine Research Center, NIAID, NIH, DHHS, Bethesda, MD, USA
156 Rupprecht, C.E., Hanlon, C.A. and Hemachudha, T. (2002) Rabies re-examined. Lancet Infect Dis 2, 327-343, PubMed: 12144896
157 (2001) Updated U.S. Public Health Service Guidelines for the Management of Occupational Exposures to HBV, HCV, and HIV and Recommendations for Postexposure Prophylaxis. MMWR Recomm Rep 50, 1-52, PubMed: 11442229
158 Massoudi, M.S., Barker, L. and Schwartz, B. (2003) Effectiveness of postexposure vaccination for the prevention of smallpox: results of a delphi analysis. J Infect Dis 188, 973-976, PubMed: 14513416
159 Baize, S. et al. (2002) Inflammatory responses in Ebola virus-infected patients. Clin Exp Immunol 128, 163-168, PubMed: 11982604
160 International Society for Infectious Diseases (2004) Ebola virus, laboratory accident - USA (Maryland), February 20, 2004. Archive no. 20040220.0550, http://www.promedmail.org
161 International Society for Infectious Diseases (2004) Ebola, lab accident death - Russia (Siberia), May 22, 2004. Archive no. 20040522.1377, http://www.promedmail.org

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press

Further reading, resources and contacts

Sanchez, A. et al. (2001) Marburg and Ebola viruses. In Fields Virology (Knipe, D.M. and Howley, P.M., eds), pp. 1279-1304, Lippincott Williams & Wilkins, Philadelphia, USA

The Centers for Disease Control and Prevention provides general information and a fact sheet on Ebola haemorrhagic fever:

http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm

Text on the original 1976 outbreak of Ebola virus:
Pattyn, S.R., ed. (1978) Ebola Virus Haemorrhagic Fever, Elsevier/North-Holland Biomedical Press, New York, USA. Text available at:

http://www.itg.be/ebola/

Related articles published recently in *Expert Reviews in Molecular Medicine*:

Bodary, P.F., Wickenheiser, K.J. and Eitzman, D.T. (2002) Recent advances in understanding endogenous fibrinolysis: implications for molecular-based treatment of vascular disorders. Expert Rev Mol Med 2002, Vol. 10, Issue 7, 1-10, PubMed: 14987391

Schneider-Schaulies, S. and ter Meulen, V. (2002) Measles virus and immunomodulation: molecular bases and perspectives. Expert Rev Mol Med 2002, Vol. 4, Issue 13, 1-18, PubMed: 14585153

Whittaker, G.R. (2001) Intracellular trafficking of influenza virus: clinical implications for molecular medicine. Expert Rev Mol Med 2001, Vol. 3, Issue 5, 1-13, PubMed: 14987372

Features associated with this article

Figures
Figure 1. Paradigm showing key events in Ebola virus pathogenesis in primates.

Table
Table 1. Comparison of disease pathogenesis of Ebola virus infection in animal models and humans.
Table 2. Summary of treatment options for Ebola virus haemorrhagic fever.

Citation details for this article

Thomas W. Geisbert and Lisa E. Hensley (2004) Ebola virus: new insights into disease aetiopathology and possible therapeutic interventions Expert Rev. Mol. Med. Vol. 6, Issue 20, 21 September, DOI: 10.1017/S1462399404008300

Accession information: DOI: 10.1017/S1462399404008300; Vol. 6; Issue 20; 21 September 2004
Published by Cambridge University Press